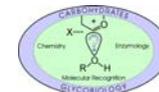# Conformational Analysis of a Tethered Disaccharide Trapped in the *anti* Conformation

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Introduction The experimental determination of the solution conformation of oligosaccharides is complicated by the paucity of distance constraints and averaging between numerous conformational micro states. The measurement of the torsional angles ϕ and ψ across the glycosidic linkage provides much needed additional information but, at natural ^{13}C abundance, the *precise* measurement of $^3J_{CH}$ coupling constants is a formidable challenge. Hence only limited model structures are available to verify the theoretically derived Karplus curve for the H-C-O-C (ϕ) and C-O-C-H (ψ) fragments, especially the higher energy *anti* conformer. Disaccharide **1** is, to the best of our knowledge, the first *unprotected* oligosaccharide trapped in the *anti* conformation. We report its conformational analysis based on NOEs, heteronuclear $^3J_{CH}$ coupling constants and molecular modeling, and include its untethered *syn* analogue, α-Gal(1-4)βGlcNAc ("α-LacNAc", **2**), for comparison.
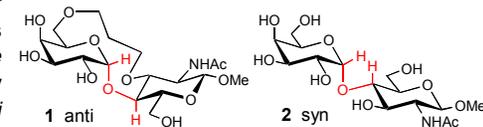
Experimental *NMR*. Excitation bandwidth selection[1] ("excitation-sculpting") as shown in Figure 1 with 5.8 ms *q3* (A) and 10.8 ms *seduce* (B) inversion pulses on the H1' and H4 (see compounds **1** and **2**) resonance, respectively. Delays based on $^3J_{CH}$ of 5.0 (A) and 9.0 Hz (B), J-scaling as indicated. *Calculations*. Biosym Discover using AMBER forcefield; only *intra*-residue constraints were applied to prevent ring flips but no NMR-derived *inter*-residue constraints were applied.

Table 1 *Inter*-proton distances (NMR based on 2.44 Å α-Gal-H1:H2 reference distance)

pyridine-D5, 27.0°C	1 anti			2 syn		
600 MHz, mix=400 ms	H1'-H3	H1'-H4	H1'-H5	H1'-H3	H1'-H4	H1'-H5
1D-TROESY (% NOE)	2.4 (6.0)	3.5 (0.7)	2.3 (7.6)	3.5 (0.6)	2.2 (9.1)	4.1 (0.2)
2D-TROESY	2.4	3.6	2.3	3.6	2.2	3.9
global minimum	2.3	3.7	2.3	3.6	2.6 *	4.6
5 ns mol. dynamics	2.3	3.7	2.4	3.7	2.4	4.4

Table 2 $^3J_{CH}$ [Hz] and torsional angles based on EXSIDE and Karplus analysis[2]

pyridine-D5, 27.0°C	1 anti		2 syn	
800 (**1**) and 600 MHz (**2**)	ϕ	ψ	ϕ	ψ
EXSIDE	4.2 -31°	7.0 -180°	4.5 -27°	4.9 -21°
global minimum	4.3 -29°	6.4 -166°	2.6 -49°*	4.4 -28°
5 ns molecular dynamics	4.2 -31°	6.2 -161°	4.7 -24°	4.7 -24°

* minimum with a carbohydrate-specific forcefield (GEGOP): 2.3 Å and -27° / 4.5 Hz.
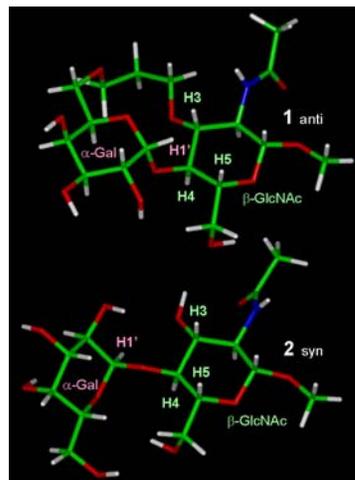
Figure 2 Global minimum structures

Results The results of T-ROESY[3] and EXSIDE[4] analysis, shown in Tables 1, 2 and Figure 1 are in very good agreement with calculated distances and torsional angles, especially for the conformationally restricted disaccharide **1**. The global minimum conformations are shown in Figure 2. Pyridine-D5 was chosen because water and methanol solutions precluded selective excitation of H4 without perturbing H3. Even in pyridine at 600 MHz this problem persisted and hence the EXSIDE spectra of **1** were acquired at 800 MHz. NOE comparisons with a methanol solution show that there are no solvent-induced conformational changes.

Of particular interest is the EXSIDE result for ψ in the *anti* conformation. The experimental result is 0.2 Hz higher than the theoretical maximum of the Karplus curve. This falls within the experimental error, however, all calculations carried out so far indicate that ψ is only about -165° or 15° below the maximum possible -180°.

Conclusion Our results indicate that the Karplus curve[2] is likely overestimating torsional angles at the *trans*-end by about 15°. This need not imply an error throughout the entire curve but suggests a systematic error in the $^3J_{CH}$ analysis of *anti* conformers.

References 1. K. Stott, J. Stonehouse, J. Keeler, T.-L. Hwang & A. J. Shaka, *J. Am. Chem. Soc.* **117**, 4199 (1995); 2. I. Tvaroska, M. Hricovini & E. Petrakova, *Carbohydr. Res.* **189**, 359 (1989); 3. T.-L. Hwang & A. J. Shaka, *J. Magn. Res. B* **102**, 155 (1993); 4. V. V. Krishnamurthy, *J. Magn. Res. A* **121**, 33 (1996).
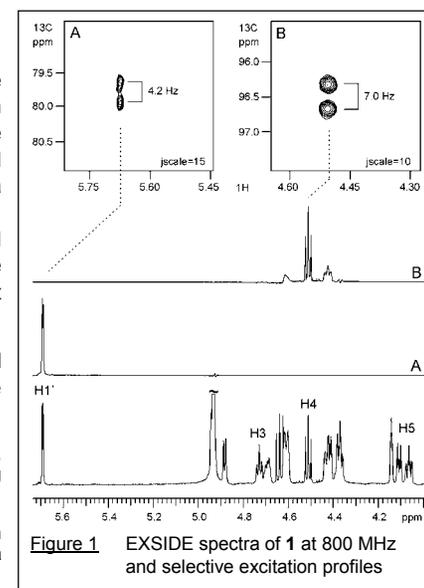
Figure 1 EXSIDE spectra of **1** at 800 MHz and selective excitation profiles